Crystal Structure of the Hexameric Assembly Unit of the HIV Capsid

The ribonucleoprotein genomic complex of human immunodeficiency virus type 1 (HIV-1) is encased within the mature capsid, a predominantly cone-shaped shell assembled from ~1,500 copies of the viral CA protein [1]. Packaging of the viral genome and its associated enzymes into the capsid is required for their delivery into host cells. To form a closed shell the CA protein assembles into ~250 hexamers. The CA hexamer consists of a ring of six N-terminal domains around a central core, surrounded by an outer ring of six more mobile C-terminal domains. Based on crystal structures of the individual domains, and electron microscopy (EM) data of assembled hexamers, the C-terminal domains are in contact with neighboring N-terminal domains by rotation of 60º about the hexamer 6-fold axis. Flexibility between these domains allows the CA hexamer to subtly adapt its shaped within the curved array of the assembled capsid.

Until now it has not been possible to crystallize the intact CA hexamer. By modeling crystal structures of the individual CA domains into a 9 Å resolution EM map of assembled hexamers [2], ten possible disulfide crosslinks were designed to stabilize interactions between adjacent N-terminal domains. Engineering of the double mutants and biochemical assay identified one crosslinked hexamer as being uniquely stable and homogeneous. This stabilized hexamer yielded large single, orthorhombic crystals diffracting to 2.7 Å at SSRL beam line 7-1; the structure was solved by molecular replacement with reference to the EM model. The crystals contained two copies of CA hexamers per asymmetric unit, providing 12 independent views of previously unknown details for the interface between N- and C-terminal domains (PDB deposition 3H4E). Analysis of the structure revealed that networks of water molecules mediate interactions between hydrogen bonding residues and sites on helices of each domain, allowing the distinct variability required for capsid formation. At the same time, comparison showed that the C-terminal domain exhibits internal plasticity in the positioning of its helices (**Fig. 1**). The structure also afforded high resolution views of the packing of N-terminal domains around the hexamer 6-fold axis, and of the 2-fold interaction between C-terminals domain as they occur in the capsid.

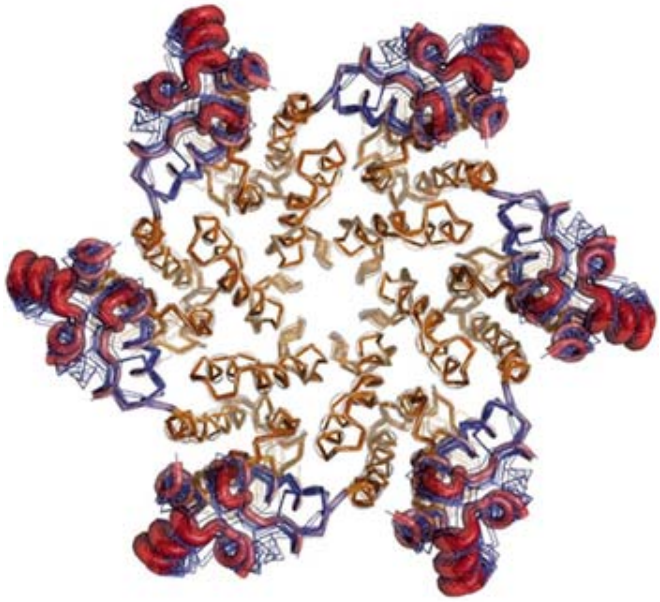

Figure 1. Superposition of multiple copies of the HIV-1 CA hexamer, showing plasticity of the smaller C-terminal domain (blue lines and red tubes) relative the N-terminal domain (brown). The C-terminal domain of one CA subunit interacts with the N-terminal domain of an adjacent CA subunit by clockwise rotation about the central 6-fold axis. Variability in the conformation and position of the C-terminal domains allows the hexamer to assembly into the curve, cone-shaped capsid of the virus.

The new crystal structure permits assembly of the intact HIV-1 capsid to be visualized at atomic resolution. Because the capsid performs an essential role early in the viral replication cycle, inhibition of capsid assembly by small molecules is a new therapeutic strategy for the treatment of HIV/AIDS. To date all FDA approved drugs for AIDS target the viral

enzymes protease, reverse transcriptase, and integrase. The N- and C-terminal domains are attractive sites for inhibition because experimental inhibitors of HIV-1 capsid assembly target them [3, 4]. New classes of drugs that target the interface of the N- and C-terminal domains to inhibit capsid assembly, or alternatively, enhance capsid stability and prevent uncoating, would provide novel means to intervene in the virus replication cycle, and important tools to combat the evolution of resistance in HIV. The mode of binding of such compounds can now be studied in detail, and new binding sites can be identified, facilitating structure-based drug design strategies.

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Primary Citation

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